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Kidney Transplantation Under a Tolerogenic Regimen of Recipient Pretreatment and Low-Dose Postoperative Immunosuppression With Subsequent Weaning

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Objective: The purpose of this work was to perform kidney transplantation under a regimen of immunosuppression that facilitates rather than interferes with the recently defined mechanisms of alloengraftment and acquired tolerance.

Summary Background Data: In almost all centers, multiple immunosuppressive agents are given in large doses after kidney transplantation in an attempt to reduce the incidence of acute rejection to near zero. With the elucidation of the mechanisms of alloengraftment and acquired tolerance, it was realized that such heavy prophylactic immunosuppression could systematically subvert the clonal exhaustion-deletion that is the seminal mechanism of tolerance. In addition, it has been established that the rejection response can be made more readily treatable by pretransplant immunosuppression. Consequently, we conducted kidney transplantation in compliance with 2 therapeutic principles: recipient pretreatment and the least possible use of posttransplant immunosuppression.

Methods: One-hundred fifty unselected renal transplant recipients with a mean age of 51 ± 15 years and multiple risk factors had pretreatment with approximately 5 mg/kg of rabbit antithymocyte globulin (Thymoglobulin) in the hours before transplantation, under covering bolus doses of prednisone to prevent cytokine reactions. Minimal posttransplant immunosuppression was with tacrolimus monotherapy to which steroids or other agents were added only for the treatment of rejection. At or after 4 months after transplant, spaced-dose weaning from tacrolimus monotherapy was begun in patients who had exhibited a satisfactory course.

Results: One-year actuarial patient and graft survival was 97% and 92%, respectively. Although the incidence of early acute rejection was 37%, only 7% required prolonged treatment with any agent other than tacrolimus. After a follow-up of 6 to 21 months, the mean serum creatinine in patients with functioning grafts is 1.8 ± 1.0 mg/dL. Seventy-three percent of the patients met the criteria for spaced weaning. Although rejection episodes occasionally required restoration of daily treatment, 94 (63%) of the 150 patients currently receive tacrolimus in spaced doses ranging from every other day to once a week.

Conclusions: With this approach to immunosuppression, it has been possible to avoid early posttransplant overimmunosuppression and thereby to promote the evolution of a degree of partial tolerance sufficient to undertake substantial dose reduction. The strategy, which is applicable for all organ grafts, constitutes a paradigm shift in transplant management at our center.

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The prophylactic postoperative administration of multiple drugs with the objective of completely preventing acute rejection is the most widely used treatment of kidney and other kinds of organ transplant recipients. With ever more potent agents used in combination, rejection rates <10% have been reported in some series, whereas 1-year renal graft survival has increased to the current level of nearly 90%.[1] Chronic rejection has become endemic, however, and there have been other disappointments. Continuous treatment with the more potent new drugs can damage recipient vital organs, cause potentially dangerous metabolic effects, and increase the risk of infections and malignant tumors that are normally kept under control by the immune system.

The ideal solution would be to make organ recipients more tolerant and thereby less immunosuppression dependent. This objective became realistic with the elucidation of the donor leukocyte chimerism-associated mechanisms of

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acquired tolerance[2-4] and the recognition that organ engraftment is a form of organ-induced partial tolerance. With this insight, it could be seen that the mechanisms of tolerance (clonal exhaustion-deletion and immune ignorance) can be subverted by the customary heavy immunosuppression.[5] It also was proposed that this undesired consequence could be prevented by observance of 2 therapeutic principles: recipient pretreatment and the use of minimal posttransplant immunosuppression.[5] We report here the outcomes in 150 patients undergoing renal transplantation under such tolerogenic immunosuppression. Details of the first 40 cases were recently reported.[6]

PATIENTS AND METHODS

The regimen of immunosuppression was submitted to the University of Pittsburgh Institutional Review Board, which determined it to be within the boundaries of standard therapy. The protocol was then remanded to the Presbyterian University Hospital Innovative Practices Committee and to the Pharmacy & Therapeutics Committee, with approval by both. All patients provided standard informed consent. In addition, separate informed consent was obtained for studies of immune parameters not routinely obtained in our conventional practice. Data integrity as well as safety and efficacy monitoring were assured by establishment of a weekly formal review of all cases.

Between July 2001 and October 2002, 150 adults underwent kidney transplantation under the described treatment regimen (Table 1). There were 93 (62%) cadaveric and 57 (38%) live donors. The mean recipient age was 51 ± 15 years (range, 19–80). Twenty-five (17%) were African-American, 15% were undergoing retransplantation, and 17% had a PRA over 20%. The mean number of HLA mismatches was 3.2 ± 1.7, and in the cadaver cases, it was 3.3 ± 1.8. Although the mean donor age overall was 40.3 ± 15.5 years (range, 0.5–66), the adult cadaveric donors were 43.8 ± 13.1 years. The mean cold ischemia time of the cadaver kidneys was 26.1 ± 6.6 hours. On the first postoperative day, 41 (27%) of the recipients received an infusion of donor bone marrow cells.[7]

Pretreatment consisted of 5 mg/kg antithymocyte globulin (Thymoglobulin; SangStat, Fremont, CA) in the few hours or the evening before transplantation, with concomitant 1-g bolus(es) as prophylactics against a cytokine release syndrome. Twice daily oral tacrolimus (Prograf, Fujisawa Healthcare, Inc., Deerfield, IL) was started on postoperative day one, with target 12-hour target trough levels of 10 ng/mL. By 3.5–4 months in suitable patients, or in some cases at a later time, the twice-daily dose was consolidated to once a day. After 1–2 more months, the dose schedule was changed to every other day (Fig. 1). After variable intervals (usually at least 2 months), the interval was changed to 3 times/week, and subsequently to 2 times/week. At about 1 year posttransplantation, selected patients who had been on twice a week tacrolimus for at least 4 months were put on once-weekly tacrolimus. Once spaced weaning was begun, trough levels of tacrolimus became low, and if they were obtained just before the next dose, they were undetectable (Fig. 1). Maintenance

TABLE 1. Graft Survival

	n (%)	Functioning Grafts (%)*	mg/dL Cr (SD)*
Living donor	57 (38%)	55 (96%)	1.81 (1.22)
Cadaver donor	93 (62%)	82 (88%)	1.87 (0.85)
Bone marrow	41 (27%)	33 (80%)	2.06 (1.54)
No bone marrow	109 (73%)	104 (95%)	1.77 (0.77)
Non-African American	125 (83%)	116 (92%)	1.81 (1.04)
African American	25 (17%)	21 (84%)	2.02 (0.83)

*At 11 ± 5.4 months.

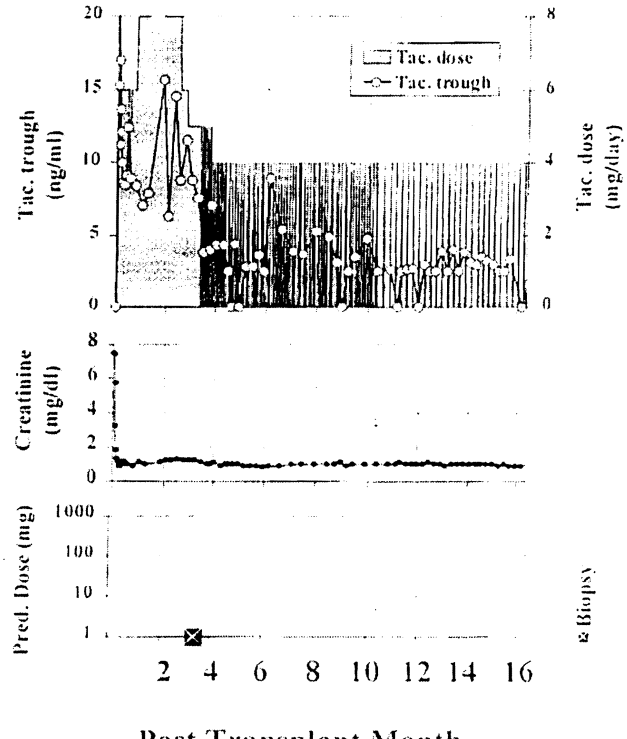

FIGURE 1. Uncomplicated postoperative course. The patient had immediate graft function and experienced no episodes of acute rejection. After a clean biopsy obtained at 90 days, weaning by stages was commenced to once-weekly doses of tacrolimus. Most of the tacrolimus trough levels during weaning were at 24 or 48 hours.

steroids were not routinely administered, nor were mycophenolate mofetil or sirolimus.

Biopsy-proven breakthrough rejections usually were treated first with a 1-g bolus of intravenous methylprednisolone. Steroid-resistant rejection was treated either with muronmonab-CD3 (OKT3) or alemtuzumab (Campath 1H, ILEX Pharmaceuticals, LP, San Antonio, TX). Maintenance steroids or sirolimus were added if necessary. Conversion from tacrolimus to sirolimus was used in cases of significant nephrotoxicity. This was performed most frequently when "expanded criteria" kidneys were used, for example, kidneys with preexisting disease (arteriosclerosis arteriolosclerosis, interstitial fibrosis, or glomerular sclerosis) or when the kidneys were from very old donors.

RESULTS

Patient Survival

After a mean follow-up of 11 ± 5.4 months (range, 6–21), the 1-year actuarial patient survival was 97%. Four (2.7%) patients died: 1 each from intraoperative volume overload (an anesthetic error), intraabdominal sepsis caused by retained peritoneal dialysis catheter, a late myocardial infarction, and inanition after a prolonged colonic infection with Clostridium difficile. None of the 4 deaths was an African-American.

Graft Survival and Function

The 1-year actuarial graft survival is 92%. Including the 4 lost from patient death, 13 (8.7%) of the transplanted kidneys were lost. Three of the 9 graft losses not associated with mortality were from a combination of preexisting donor disease and chronic rejection. The other 6 were caused by cortical necrosis after an intraoperative Shwartzman reaction,[8] renal artery thrombosis during cardiopulmonary bypass and coronary artery reconstruction for an early postoperative myocardial infarction, a surgical/technical error, the inability to arterialize the renal graft because of recipient peripheral vascular disease, primary graft nonfunction, and thrombosis of a set of en bloc kidneys from a 6-month-old cadaveric donor.

Graft survival in the live versus cadaveric, adjunct bone marrow, and African-American subgroups is shown in Table 1. It is noteworthy that African Americans were underrepresented versus all others in the optimal live donor cases: that is, 4/25 (16%) versus 53/125 (42.4%). In contrast, they were overrepresented in historically disadvantaged retransplantations: 5/25 (20%) versus 17/125 (13.6%). Finally, 11/28 (44%) African-Americans had adjunct donor bone marrow infusion versus 30/125 (24%) for all others. Although it is too early for a definitive multivariate analysis, ethnicity per se may or may not have been the dominant factor in the poorer graft survival and function in both the cadaver kidney and adjunct bone marrow subgroups.

The mean serum creatinine of the 137 surviving kidneys is 1.8 ± 1.0 mg/dL. The analysis of subgroups is shown in Table 1. The incidence of delayed graft function, defined as the requirement for dialysis in the first week after transplantation, was 23%, reflecting the systematic use of "marginal"

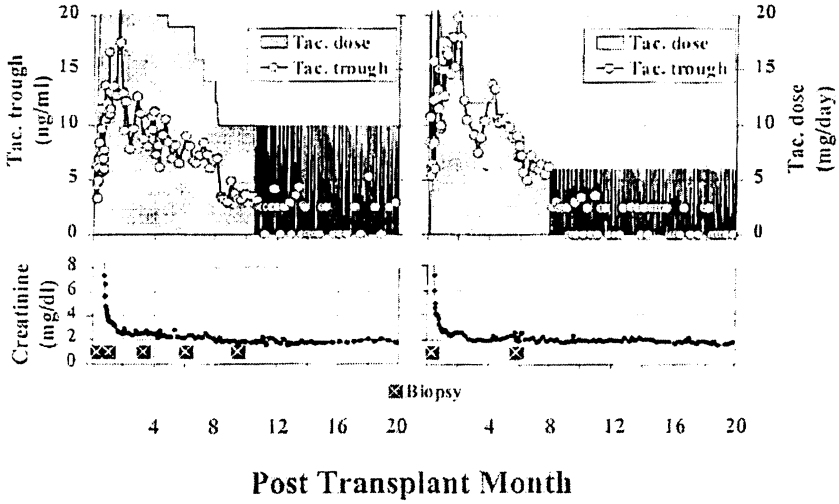

FIGURE 2. Delayed graft function necessitating postoperative dialysis support for 1 month of 2 cadaver kidneys transplanted from the same older donor in July 2001. Management was dictated by biopsies that showed only ischemia reperfusion injury. Although 1 patient received donor bone marrow, convalescence in the 2 cases was similar. Weaning was delayed well beyond 4 months to allow completion of the slow decline of serum creatinine concentration before altering therapy. Both patients ultimately were weaned to twice-weekly doses of tacrolimus. Note that the individual tacrolimus doses after spaced weaning were the same as those before.

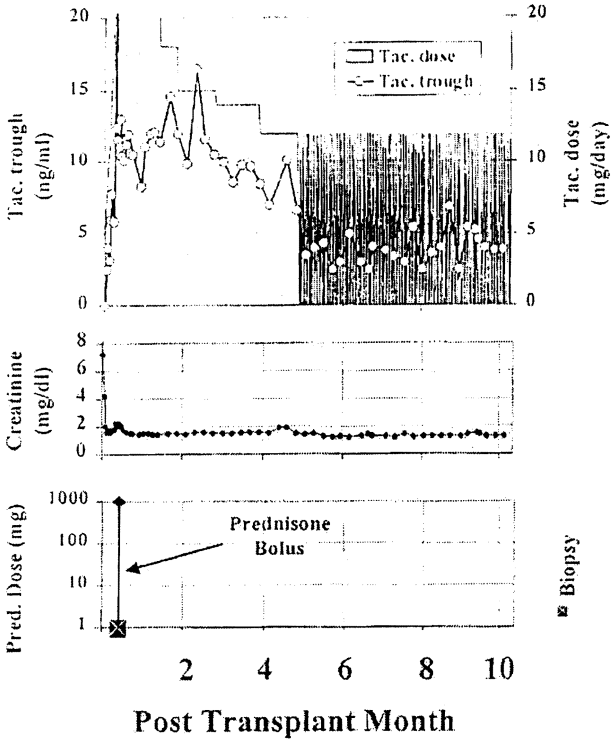

FIGURE 3. A single episode of early acute rejection was treated with a single bolus of steroids. Every-other-day spaced weaning was begun at 5 months. The patient now receives 3 doses/week of tacrolimus.

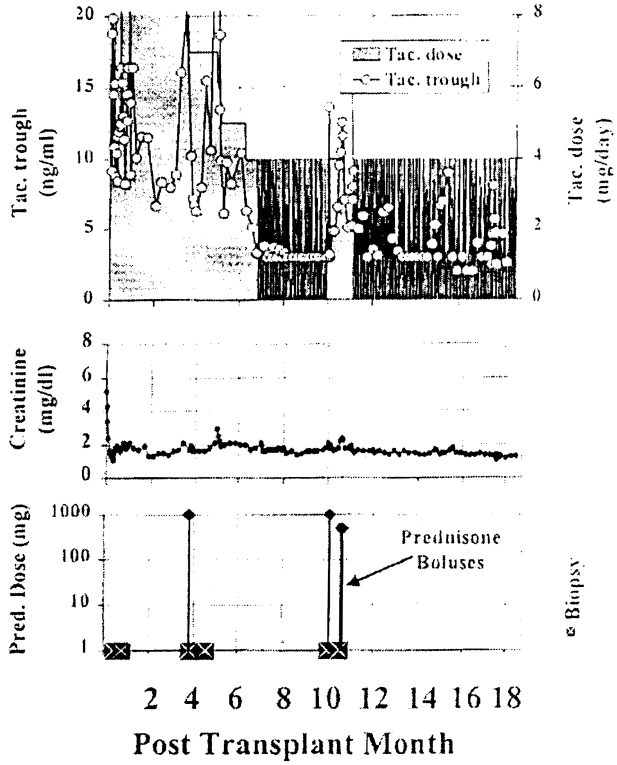

FIGURE 5. Late rejection in a 73-year-old cadaver kidney recipient after reduction of the frequency of tacrolimus doses from 3 times/week to twice weekly. Two steroid boluses (1 g and 0.5 g) and resumption of daily tacrolimus restored baseline kidney function. Tacrolimus doses were subsequently weaned to every other day and are now being given 3 times/week.

cadaver donor kidneys. Management of these cases was not different than for the other patients except for greater dependence on early posttransplant biopsies (Fig. 2).

Incidence of Preweaning Acute Rejection

Prior to weaning, the incidence of clinical acute rejection, diagnosed by biopsy and given a Banff score of 1A or higher[9] in the context of a rising serum creatinine, was 37% (55 of 150). However, most of these rejections were readily reversed with a single 1 g bolus of prednisone (Fig. 3). The incidence of steroid resistant rejection was only 7.3% (11 of 150).

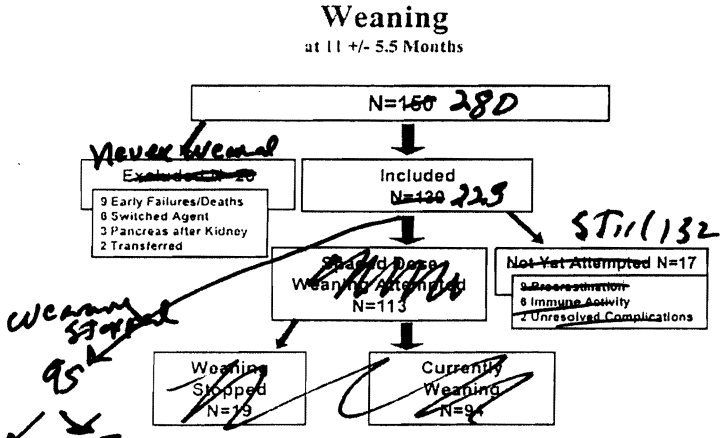

FIGURE 4. Rate of weaning and reasons for not weaning in 150 kidney transplant recipients.

Spaced Weaning

Weaning was precluded in 20 cases, in 4 because of patient death and in 5 more because of early graft failure (Fig. 4). Other exclusionary factors were discontinuance of tacrolimus because of toxicity (n = 6), transplantation of a pancreas after kidney (n = 3), or transfer of 2 recipients to other cities where care givers instituted multiple drugs. A decision against weaning was made in 8 more cases because of an unstable course (Fig. 4). Finally, weaning has not been instituted in 9 recipients because of hesitation of patients in taking this step or because of procrastination by our management team.

Spaced weaning was begun in 113 of the 150 recipients. Twenty-six (23%) of the 113 weaned patients experienced clinical acute rejection at some point subsequent to the

institution of weaning. Treatment ranged from a bolus of steroids and/or a temporary increase to once daily dosing of tacrolimus, to antibody therapy with muromonab-CD3 (OKT3) or alemtuzumab (Campath 1H). Subsequent reinstitution of spaced weaning was often possible (Fig. 5). At present, 94 (63%) of the original 150 patients (70% of the 137 with functioning kidneys) are on every other day (n = 35), 3 times a week (n = 29), twice a week (n = 19), or once-weekly (n = 11) tacrolimus.

Nonrenal Morbidity

Posttransplant diabetes mellitus was observed in 1 (0.7%) patient who had sirolimus and prednisone added to once daily tacrolimus for a late rejection. The patient was able to discontinue insulin after the sirolimus and prednisone were stopped, and reinstitution of tacrolimus weaning to every other day. Posttransplant lymphoproliferative disorder (PTLD) was not observed. Cytomegalovirus was seen in 3 (2%) recipients, who were treated successfully with oral valgancyclovir and tacrolimus dosage reduction. One (0.7%) patient developed evidence of BK (polyoma) virus. He had been on a 3 times/week dose schedule for tacrolimus and was treated by reducing tacrolimus to 1 dose/week and intravenous cidofovir.

DISCUSSION

The strategy of immunosuppression described here was used in a consecutive and unselected series of kidney transplantations in which there were multiple risk factors: the systematic use of "expanded criteria" cadaveric kidneys, many older age recipients, a high incidence of retransplantation and/or presensitization, long cold ischemia times, and generally poor HLA matches. Nevertheless, good 1-year patient (97%) and graft (92%) survival rates were obtained. Nearly two thirds of the patients could be weaned to extremely low levels of maintenance immunosuppression. Consequently, the overall freedom from the morbidity of chronic immune depression per se and from the organ-specific toxicity of the individual drugs was striking. Although weaning resulted in a 23% incidence of acute rejection, these were readily controlled, often with the subsequent resumption of weaning. There was very little immediate or subsequent morbidity.

Our experience suggests that the quality of outcome with kidney transplantation can be substantially improved by simplifying immunosuppression and above all by modifying its timing and intensity. However, a drastic change in the intellectual culture of transplantation will be required. Until now, the development of immunosuppression protocols has been driven by the conviction that complete prevention of acute rejection is a genuine measure of therapeutic efficacy. Contrary to this belief, rejection and tolerance in the concept from which our treatment algorithm derived[4,5] are simply alternative outcomes of the immune activation that is induced by the migration of donor passenger leukocytes to recipient lymphoid organs.

In this view, the seminal mechanism of alloengraftment and of acquired tolerance is donor leukocyte-driven exhaustion and deletion of the antidonor immune response.[2-5] The purpose of pretreatment is to reduce global immune responsiveness in advance far enough so the antidonor response induced by the graft's passenger leukocytes is brought into a more deletable range. The objective of minimal posttransplant immunosuppression is avoidance of depression of the donor specific immune activation to such an extent that the activation-dependent event of clonal exhaustion-deletion is eroded or precluded.

Of historical interest, a strategy incorporating both the principle of recipient pretreatment and the principle of minimalistic immunosuppression was used in 1962–1963 at the University of Colorado[10] for the generation of a bellwether series of long-surviving kidney allograft recipients that established renal transplantation as a clinical service.[11] The recipients of kidneys from 46 live related donors were pretreated with azathioprine for 1–2 weeks before transplantation and then given azathioprine monotherapy afterward. Prednisone was added only for the indication of overt rejection. Nine of the kidneys subsequently functioned for the next 4 decades, and are the longest surviving organ allografts in the world.[12] Importantly, 7 of the 9 patients became drug-free tolerant for periods of 3–38 years.[13]

No similar cluster of tolerant kidney recipients was produced subsequently, anywhere in the world. With delineation of the mechanisms of organ engraftment and acquired tolerance, the explanation for the failure to duplicate these results was evident. In December 1963, pretreatment was de-emphasized because a significant number of immunosuppression-related infectious complications had occurred prior to transplantation. A second modification was prompted by losses of kidney allografts whose rejections could not be reversed or controlled once they had begun. In a violation of the principle of minimal posttransplant immunosuppression, high doses of prednisone were now instituted from the time of operation, rather than as specifically indicated (see * with Reference 11).

The policy of prophylactic high dose early immunosuppression with multiple agents (inappropriately called "induction") has dominated the practice of transplantation ever since with a few exceptions that have been summarized elsewhere.[13] In the most recent exception, Calne et al[14] treated cadaver kidney recipients with a few perioperative doses of the broadly-reacting humanized monoclonal antibody, alemtuzumab, followed by low daily maintenance doses of cyclosporine monotherapy. The authors characterized the trouble-free convalescence of their patients by the term "prope tolerance." We believe that Calne's results reflected

the efficient production of genuine partial tolerance. More recently, Swanson et al[15] and Knechtle et al[16] have demonstrated the feasibility of kidney transplantation under sirolimus monotherapy in patients pretreated for a week with large doses of thymoglobulin or with alemtuzumab (Campath 1H) given intraoperatively and on postoperative day 1.

The archival and more recent observations with different immunosuppressants, as well as our own current experience reported here and elsewhere,[6] suggest that the therapeutic principles of our protocol are neither drug-specific nor organ allograft-specific. We began with the use of Thymoglobulin for pretreatment because it is a well-standardized and broadly reactive polyclonal antilymphocyte globulin with a long record of use in transplantation. The monoclonal antibody alemtuzumab has similar qualities and has been used electively in many of our recent cases. For posttransplant monotherapy, the calcineurin inhibitor, tacrolimus, is a superior agent, but weaning from cyclosporine and sirolimus also was possible in some of our cases.

Although none of our patients have had total discontinuance of immunosuppression, the development of donor specific tolerance is implicit in the ability to wean the majority of recipients to every other day, 3 times a week, twice a week, or even once per week dose schedules. Moreover, the evolution of high, if not absolute, levels of tolerance has been formally demonstrated with limiting dilution, mixed lymphocyte reactivity, and cytokine assays in some of the first patients treated with the current protocol as has been reported elsewhere.[6]

Much longer follow-up of this protocol will be required to determine whether the high incidence of acute rejection is deleterious to long-term graft function and survival. However, the observations to date appear to presage a new era of transplantation in which recipients can aspire to a better quality of posttransplant life. Unlike previous improvements in care, this one will not be primarily dependent on the development of more powerful immunosuppressive agents.

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Discussion

DR. JOHN S. NAJARIAN (Minneapolis, Minnesota): Thank you, Dr. Shapiro, for an excellent presentation of a considerable amount of data from the Pittsburgh group – as usual, very well presented. I also enjoyed reading the corresponding paper, which I just received before your presentation.

I would just like to make an initial comment. Dr. Matas at our institution has concluded a clinical trial of a steroid-free immunosuppressive protocol for 300 kidney transplant recipients with a 3-year follow-up. The steroids were rapidly decreased; by postoperative day 6, recipients were no longer on them. Under this protocol, the total rejection rate was 6%; only 1% of those episodes were steroid resistant. In contrast, most immunosuppressive protocols today result in acute rejection rates of close to 12%.

Under your 'tolerogenic regimen,' you reported an initial 37% acute rejection rate. Then after weaning your recipients off of immunosuppression, another 23% of them experienced acute rejection. Both these numbers seem to be very high. A decade ago, we showed that it takes 3 to 5 years before it is possible to determine how many kidney recipients with acute rejection will go on to chronic rejection. So, your conclusion regarding the early nature of your study is very important. Until you get late results, it will be difficult to understand the meaning of your study. It is an interesting concept, though, to get down to monotherapy. All of us will be intrigued to hear the late results of your study. I was perplexed by the title of your presentation, which used the word 'tolerogenic.' But since none of your recipients became tolerant, I am wondering what you actually meant by 'tolerogenic' in the title. It caught my eye as I read the abstract in the program booklet.

Another interesting aspect of your paper is that many different investigational strategies were used for these recip-

ients. There was no single protocol. Some recipients received cadaver kidneys; others, living donor kidneys. Some received antibody pretransplant; others, Campath; and still others, donor bone marrow. You said that all recipients did about the same, but I would be curious to know whether the bone marrow recipients, when looked at individually, did any better than the nonbone marrow recipients.

Another question concerns primary transplant recipients who were nonsensitized: were their results any different from the results of retransplant recipients who were sensitized? Also, did you keep the same dose of tacrolimus all the way through, even though you sometimes reduced it to twice or even once a week (maintaining it at 1 microgram per liter)? Or did you just let the patients continue on the same dose?

Finally, this could prove to be an important study and I certainly look forward to your long-term results. But again, I am concerned about the excessively high acute rejection rate that you have reported. As you know, it is important to see whether or not such recipients are eventually going to be subjected to chronic rejection; if so, 37% initially and 23% after weaning are unacceptable levels of acute rejection. Thank you.

Dr. Ron Shapiro (Pittsburgh, Pennsylvania): Thank you, Dr. Najarian. These are all excellent points.

The rejection incidence reflects a philosophic belief that you do need to have some sort of donor and recipient interaction. It is thought that if you can keep this under some control that you can then get on with subsequent weaning. As I showed in the slide with the patient that did have a little bit of rejection, it was very easily controlled with a single bolus of steroids, and then we were able to continue to wean him.

Parenthetically, in some of our more recent cases we have been using Campath IH 30 mg as preconditioning, usually because we do not have the time to give the full dose of Thymoglobulin, and the incidence of rejection in that series seems to be incredibly low, less than 5%. One of the questions, in fact, will be whether it will be too low and we will find that we will not be able to wean patients. I will let you know as we get more follow-up on these patients.

I agree with you that these are not formally tolerant patients. On the other hand, the doses of immunosuppression that they are receiving bear no relationship to anything I would have ever predicted 2 years ago, with many patients taking once a week or twice a week immunosuppression.

It is a relatively homogeneous protocol in that patients do get preconditioning with 5 mg/kl of Thymoglobulin and received tacrolimus monotherapy aiming for levels of about 10. Weaning then tends to follow at reasonably regular intervals, beginning at about 4 months, with some variations.

The subgroup analyses are still a little bit too early to perform, as the numbers are quite small, and I cannot really comment in terms of actuarial survivals. But when we did a first-pass look at some of these issues, we did not see any significant differences. The bone marrow patients did not do better or worse statistically than the patients who did not receive bone marrow. In fact, for cadaveric cases now, while we are going to continue to follow the patients who received donor specific bone marrow, we are not adding new cases at the present time. Again, while we do not have sufficient follow-up on the patients undergoing retransplantation, it does not appear as though there were significant differences in outcomes.

The dosing aims for levels of about 10 mg/ml at the beginning for the first 3 or 4 months. Once we go to once-a-day immunosuppression, we consolidate the twice-a-day dose to once a day. So someone who is taking 4 mg twice a day will go to 8 mg once a day. We will then use the 8 mg dosing as we wean, going to every other day, 3 times a week, twice a week, and of course at that point the levels become essentially undetectable.

Dr. James A. Schulak (Cleveland, Ohio): I, too, share many of the concerns that Dr. Najarian voiced about this paper, particularly the high incidence of rejection.

I think we all know that early rejection episodes often are predictive of the development of chronic rejection and poor long-term outcomes. I encourage you to watch these patients very carefully to see if that is happening and intervene if it appears to be occurring.

The hypothesis that you are testing is that the immune system after receiving an allograft is a pendulum that can swing either in favor of rejection or toward tolerance. You also suggest that your potent lymphocyte depleting regimen followed by low dose immunosuppression with tacrolimus favors the latter. Did you do any type of immune function monitoring in these patients afterward that indeed suggests that something of this nature is occurring? I think it is very important for you to do so if you are going to make the statement that this regimen does cause tolerance to occur.

I think that the analysis of all of your patients as 1 big group needs to be addressed. You lump live donor with deceased donor transplants as well as patients of all ethnicities and both genders. I think it is extremely important that you do cohort analysis if we are going to learn more about this phenomenon, and I am wondering if you have done that.

Finally, my last question is, do you really think the high dose antithymoglobulin is necessary? Might we all achieve the same types of results using any of the protocols we currently use in our centers if we just try?

Dr. Ron Shapiro (Pittsburgh, Pennsylvania): Thank you, Dr. Schulak.

Again, the incidence of rejection I have addressed. It looks as though by using Campath IH preconditioning we

may see much less rejection, although most of the early rejections under Thymoglobulin tend to respond to relatively little modification of immunosuppression.

We are we are doing active immune monitoring. We do not have the outcomes on that yet.

The subgroup analysis is in fact important. We have tried to take a first look at it. We have not seen anything that has been particularly remarkable looking either at living donor versus cadaver outcomes, African-Americans versus non-African-Americans, or bone marrow versus nonbone marrow. However, we need more patients and more follow-up to provide that level of detail.

I think that you probably do need some sort of preconditioning to be able to wean to such incredibly low doses of immunosuppression. I believe that on theoretical grounds you need to do that.